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Comparative Biochemical Study of Glutathione, Ceruloplasmin and Trace Element in Sera of Control Group and Human Female Patients with Osteoarthritis Nodal in Iraqies Patients

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Abstract

The present study conducted on 30 female patients with osteoarthritis 0A a attending Baghdad teaching hospital, in addition to 30 healthy females , all subjects were (35-65) years old.

Some biochemical parameters were measured in the sera of patients and healthy groups. The parameters were Glutathione (GSH). Ceruloplasmin (Cp) and some trace elements ,including Copper (Cu) ,Cu/ Cp ratio and Selenium (Se) were determined . The results revealed a significant reduction in all parameters of patients sera compared to healthy group.

The reduction in GSH and Cu/Cp ratio confirms tissue damage associated with oxidative stress injury

A conclusion was obtained hrer ,that Cu wasn't an important element in oxidative stress in patients .

Introduction

Osteoarthritis (OA) is a slow progressive disorder of synovial joints .This joints disorder is characterized by a loss of balance between synthesis and degradation of the articular cartilage constituents leading to subsequent erosion of joint cartilage remodeling of the underlying bone oseophyte formation and variable degree of synopitis [1].When clinical characteristics of OA (pain loss of mobility and radiographic narrowing of the joint space) manifest , the actual changes in articular cartage and subchondrla ,bone have started long ago[2]

Glutathione (γ-glutamyl cysteinyl glycin) is atripeptide commonly abbreviated GSH. It is the most common intracellular thiol which is found in most mammalian cells as an important defense mechanism against certain toxic compounds such as some drugs and carcinogens. If these toxic electrophiles (xenobiotics) were not conjugated to GSH, they would be free to combine covalently with DNA, RNA, or cell protein and could thus lead to serious cell damage [3]. If the levels of GSH in a tissue are lowered than that tissue can be shown to be more susceptible to injury by various chemicals that would normally be conjugated to GSH [4].

Ceruloplasmin (Cp) is an alpha 2-glycoprotein with enzymatic activity containing six or seven copper atom per molecule ' thus Cp considered as antioxidant through its ferroxidase activity by playinga role in the oxidation of ferrous ion and hence releases from cells and loading on to apotransferrin binds largely the ferric form , so Cp is involved primarily in maintaining iron homeostasis and preventing iron mediated free radicals injury [5,6]. Copper is an essential trace element present as an integral component of many

metalloenzymes involved in oxidation reduction reactions Copper is necessary for the formation of the blood cells and connective tissue [7].

Selenium (Se) is an essential element for a human , helps to defend the organism against oxidant stress and protect and help to prevent some forms of cancers and heart disease , and also boost the immune system[8].

Aim of the study:-

The aim of this study is to evaluate some biochemical parameters such as Ceruloplasmin, Glutathione and some trace elements for their importance in oxidant- antioxidant balance in sera of OA female patients which may play a role in oxidative stress .

Experimental

Subjects

The present study was performed on a group of 30 human females aged (35-65) years with OA diagnosed by examining the patients by in the (Baghdad teaching hospital)and by x-ray examination. In addition a group of 30 healthy females were enrolled in the study as control group.

Sampling

Blood samples of 5 ml were drawn all from subjects enrolled in the study , and kept ,in plain tubes, left to clot at room temperature for l5 min. Then centrifuged at 3500 rpm for 10 min to separate the serum.

Glutathione (GSH) determination :-

Glutathione concentration in the sera of all subjects was performed according to Ell man(1959) [9] .The method is based on the reaction of aliphatic thiol compounds with 5,5- dithiobis (2-nitrobenzoic acid) (DTNB) at pH 8. The absorbance of the yellow chromagen was measured at 412 nm and is directly proportional to GSH concentration. So one mole of thiol produces one mole of p-nitrothiophenol anion which is highly colored (ϵ=13600M^{-4} Cm^{-1})

Ceruloplasmin (Cp) determination:-

The method for determination of Cp concentration in serum based on the catalytic ability of Cp to oxidize the colorless p-phenylene diamine to a blue-violet oxidized form which has a maximum absorbance at 525 nm , using molar absorbtivity coefficient of 0.68 mol^{-1} cm^{-1} for the base[10].

Determination of Copper (Cu) concentration:-

Flame atomic absorption (Shimadzu-A-A-600atomicabsorption/flameless)
was used with a hollow cathode lamp of 324.8nm.

Determination of Selenium (Se) concentration :-

Flameless atomic absorption was used with a hollow cathode lamp of 196.00 nm.

Statistical analysis:-

The results were expressed as mean ±SD of mean , using student-t-test, Significant variation is considered significant when p- values is ≤ 0.05.

Results and Discussion

The results of serum GSH and Cp of OA patients and healthy females are shown in table (1).

The results of GSH concentration in sera of OA patients were significantly lower than that for control .

This results are in agreement with other studies which reported that GSH is the major non enzymatic antioxidant in plasma and erythrocytes [11]. So this reduction is an indicative of an increased oxidative stress in patients with osteoarthritis disease , also GSH maintains the sulfhydryl groups of other molecules including enzymes in the reduced form for their proper function so the glutathione will oxidized (GSSG) , which will be no longer of great value in protection and maintaining other molecules in their native structure [12].

It have been reported that oxidative stress seems to play a role in OA, so human cartilage in patient with OA was significantly deficient in enzymatic antioxidant which are the major free radical scavengers [13] .

The life Extension foundation believes that people with OA should maintain a healthy intake of antioxidant and other supplements that support glutathione levels such as N- acetyl cysteine [14]

From table (1)also a significant decrease in Cp sera levels in OA female patients compared to control was found. It had been reported that Cp is, classified as one of the acute - phase reactants , its synthesis and secretion is altered by inflammation [15].

The reduction in sera Cp levels of OA patient in this study could be due to that the patients are of anti -inflamanrtory OA with moderate to severe pain [16].

Table (2) shows the Copper and Selenium levels in sera of OA patients and control.

The significant decrease in Cu concentration in sera of OA patients compared to control was noticed . This reduction in Cu concentration could be explained by the fact that generally serum Cu and Cp concentration are usually closely correlated with each other , because ceruloplasmin is the major copper binding proteins in serum [17] . The serum Cu/Cp ratio in OA patient group are significantly decreased in comparison to that of control group .

Since the Cu/Cp ratio reflect the concentration of copper that is bound to the albumin and free copper which is the form considered as one of most important reasons for free radicals formation in the body , so the slight decrease in Cu/Cp ratio may be a defense mechanism against many diseases [18]. The results of lower serum Cu concentration of OA patients in this study reflects that the contribution of free copper in the formation of free radicals in the patient group is not significant .

A significant decrease in Se concentration in sera of OA patients compared to control was found. The result of the present study agrees with other reported data stated that se deficiency has been correlated with a higher risk and severity of OA [19].

The concentration of Se in serum is highly responsive to the Se level in the diet [20], other reason for the decreased Se serum levels in OA patient is that Se is a component of the enzyme glutathione peroxidase at the active site (as selenocysteine) [4].

The catalyzing action of glutathione peroxidase in removing H_2O_2 from the biological system is of great importance , since accumulation of H_2O_2 may decrease the life span of the erythrocytes and importance molecules in human cells by causing oxidative damage to the cell membrane of the biological molecules [21].

From the present study a conclusion could be stated , that decreased of Cp concentration in sera of OA patient is due to oxidative stress. Also non Cp copper, as indicated by the ratio Cu/Cp seems to play no role here in the formation of the oxidative stress.

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Table (1): Glutathione and Ceruloplasmin levels in sera of OA patient and control

	control n=30	OA patient n=30	P value
GSH mM/L mean ±SD	2.50± 0.10	1.58±0.01	<0.05
Cp mg/dl mean ±SD	24.25± 2.35	18.97±3.20	<0.05

Table(2): Copper and Selenium level in sera of OA patient and control groups

	Control n=30	OA patient n=30	P value
Cu ppm mean ±SD	0.39± 0.04	0.13±0.01	<0.05
Se ppm	0.23 ±0.04	0.17± 0.05	<0.05
Cu/Cp ratio	0.016	0.006	<0.05

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية المجلد23 (2) 2010

دراسة مقار نة للمتغيرات الكيمو حيوية للكلوتاثيون والسيريولوبلازمين وبعض العناصرالنزرة في مصل مجمو عة نساء أصحاء ومريضات بالتهاب المفاصل العقدي في العراق

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الخلاصه :-

تضمنت هذه الدراسة 30 مريضة بالتهاب المفاصل العقدي اخذت من مستشفى بغداد التعليمي فضلا" عن 30 من النساء الأصحاء ، وتراوحت اعمار المجموعتين بين 35–65 سنة، وقيست بعض المتغيرات الحيوية في مصل ا لمجموعتين . وكانت المتغيرات هي الكلوتاثيون والسيريولوبلازمين وبعض المعادن النزرة مثل النحاس ونسبة النحاس إلى السيريولوبلازمين و السلينيوم .

جميع النتائج عكست فرق إحصائي معنوي محسوس في جميع المتغيرات في مصل نساء المجموعتين.وان نسب النحاس على السيريولوبلازمين يعزو لضرر الانسجة بسب جهد الاكسدة وان النحاس الحر لم يكن عاملاً مهماً في هذا الضرر.